

**Mystery seeds: Deer Mouse (*Peromyscus maniculatus*) foraging preferences on seeds of
herbaceous plants**

BIOS 35502-01: Practicum in Field Biology

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2018

Abstract:

Very often herbaceous plants community gets overlooked when it comes to seed predation studies. This study analyses the foraging preferences of deer mice, *Peromyscus maniculatus* (Wagner, 1845) on two seeds of herbaceous plants: Smooth Solomon's Seal, *Polygonatum biflorum* ((Walter) Elliot, 1817) and False Solomon's Seal, *Maianthemum racemosum* (Linnaeus) Link, 1821); which are two of the most common herbaceous plants at the University of Notre Dame Environmental Research Center (UNDERC). We hypothesized that mice would eat the herbaceous seeds; and that they would prefer *M. racemosum* given that it does not contain toxic compounds. There were statistically significant differences for the latency time between *Helianthus sp.* and *P. biflorum*. Also, there were statistically significant differences for amount of seeds eaten between *Helianthus sp.* and both *P. biflorum* and *M. racemosum*. *P. maniculatus* preferred *M. racemosum* over *P. biflorum*, which could be explained by the presence of an anthraquinone on *P. biflorum*.

Key words: Deer Mouse, *Peromyscus maniculatus*, Smooth Solomon's Seal, *Polygonatum biflorum*, False Solomon's Seal, *Maianthemum racemosum*, herbaceous plants, foraging, seed predation, anthraquinone, UNDERC.

Introduction:

Seed predation is an animal-plant interaction that has evolved at various levels (Janzen 1971). This interaction can be beneficial for both the seeds and the animal: it benefits the seeds when the animal does not eat the seeds and just moves them (seed dispersal), and it benefits the animal when they consume seeds which are rich in nutrients and energy (Vander Wall 2010; Cramer 2014). Although seed dispersal benefits plants, this is incidental on the part of the animals, for example, when they bury the seeds and then forget them (Vander Wall 2010). Seed predation has very important consequences. For example, post-dispersal seed predation is one of the factors that influences plant demography of a specific place (Hulme 1998).

The deer mice are small, nocturnal mammals, which makes visual observations of their behavior in the wild difficult (Jameson 1952), even though they are common in the northern forests of the United States (Kurta 2017). *Peromyscus maniculatus* are diet generalists but seeds make up a great part of their diets (Drickamer 1976). In fact, peaks in their populations change in proportion to seed availability (Falls et al. 2007). These rodents have a diet that varies from seeds and fruits to arthropods and fungi. However, the proportions of what they ingest can vary according to season and food availability (Jameson 1952). Nonetheless, in forest ecosystems, conifer seeds, berries and nuts are very important to this species (Pergams and Nyberg 2005; Falls et al. 2007; Stephens et al. 2014; Kurta 2017).

Taking into consideration that herbaceous plants are often overlooked and that few studies investigate seed predation on herbaceous plants, we posed the question: would deer mice eat herbaceous seeds? If so, which species would they prefer? Smooth Solomon's Seal (*Polygonatum biflorum*) and False Solomon's Seal (*Maianthemum racemosum*) are two of the most common herbaceous plants at University of Notre Dame Environmental Research Center

(UNDERC) (Flagel, *pers. comm.*) and we would like to know which of them are preferred by *Peromyscus maniculatus*, a common seed predator.

The two plant species (*Polygonatum biflorum* and *Maianthemum racemosum*) belong in the order Asparagales (Link, 1829), the family Asparagaceae (Juss., 1789), and the subfamily Nolinoideae (Burnett, 1835). Both *Polygonatum sp.* and *Maianthemum sp.* were originally in the Liliaceae (Juss., 1789) family under the Liliales (Perleb, 1826) order (The Angiosperm Phylogeny Group 1998). However, they were reclassified under the Ruscaceae (Spreng., 1826) family (that was under the Asparagales order) (The Angiosperm Phylogeny Group 2003) and then the Ruscaceae family was converted into a subfamily (Nolinoideae) of the Asparagaceae family (The Angiosperm Phylogeny Group 2009). The *P. biflorum* fruits are round pulpy berries with globose or subglobose seeds (Flora of North America Editorial Committee 2002). Each berry contains 3 locules (which are the cavities of the ovary) and each locule contains 1-2 seeds (Fernald 1950). The flowering of *P. biflorum* occurs during the spring (Flora of North America Editorial Committee 2002) and the fruiting occurs from May to late June (Fernald 1950). On the other hand, *M. racemosum* fruits are round, usually lobed, thin-pulped berries with globose seeds. The blooming of this species usually occurs during mid spring and the fruiting from early summer to autumn (Flora of North America Editorial Committee 2002). Although they belong to the same family, the *Polygonatum sp.* berries contain an anthraquinone (a naturally occurring organic compound) that causes vomiting and diarrhea (Flora of North America Editorial Committee 2002). This particular characteristic may make the *M. racemosum* seeds preferable to the *P. biflorum* seeds. Thus, mice should reject *P. biflorum* seeds and express a preference for *M. racemosum* seeds.

The purpose of this study was to evaluate the foraging preferences of *P. maniculatus* on the seeds of two herbaceous plants: *P. biflorum* and *M. racemosum*. We tested two hypotheses: (1) *P. maniculatus* would eat herbaceous seeds, and (2) *P. maniculatus* should choose seeds based on them not containing toxic compounds and consequently should show a preference for *M. racemosum*.

Materials and Methods:

Site of Study:

Experimental procedures were conducted at the University of Notre Dame Environmental Research Center (UNDERC). UNDERC encompasses approximately 7500 acres on both sides of the state line between Wisconsin and Michigan's Upper Peninsula. Its biomes include deciduous forests and mixed deciduous/coniferous forests. Mice used for the study were trapped at several grids around property where sugar maple trees were dominant and inside buildings where mice were also common.

Trapping:

During July, we used Sherman live traps which were baited with sunflower seeds and millet to trap the deer mice. After being caught, the deer mice were identified to species (based on their ear lengths (Ridenhour and Cramer 2015)), sexed, weighed and tagged (unless previously caught).

Experimental procedure: Obtaining berries and seeds

When the plant species started fruiting in July, developing berries from each of the plants were collected in the field. In order to count the *P. biflorum* seeds, the berries had to be cut to

take the seeds out since one cannot know how many seeds there are inside each berry. Berries from this species were meticulously dissected under a dissecting microscope to get the seeds on the same day of each trial. Dissection of the *M. racemosum* berries was not necessary since the berry has the form of the seed and the amount of seeds can be told by looking at the fruit due to it being thin-pulped.

Experimental procedure: Trials

Individuals were taken to the lab to test their feeding preferences directly. After settling them in individual cages, they were fed and given water and five hours prior to testing, food was removed. Removing the food in the late afternoon allowed an adequate starvation period that did not affect the mice due to their nocturnal foraging activities. After five hours, each individual was moved into a new cage with a petri dish (taped to the cage's bottom) divided into four sections. Three sections contained a seed/berry species. Mice were given 10 seeds of *P. biflorum*, 10 berries of *M. racemosum*, and 10 sunflower (*Helianthus sp.*) seeds. The sunflower seeds were provided to demonstrate that mice were responding adequately to the starvation period since *Peromyscus maniculatus* are known to eat these seeds. Right after, mice were left to forage while being recorded with the lights turned off for an hour. After the hour had passed, mice were removed from the trial cage and placed back into their housing cage. Thus the trial cage could be examined for seeds and/or berries left over and these could be counted. All trials were recorded to get the latency times of the seeds given.

Statistical analyses:

All statistical analyses for this research were performed using R Studio. A Shapiro-Wilk test of normality was performed to verify that the data was normal. Then, we did a Bartlett test of

homogeneity of variances to see if the variances between the groups were the same. After that, we did a one-way ANOVA to see if there were any statistically significant variances between the latency times (time elapsed before the individual noticed each seed type) in function of the seed type given. Another one-way ANOVA was performed to see if there were any statistically significant variances between the amount of seeds eaten in function of the seed type given. To evaluate these variances (if statistically significant), a post hoc test (Tukey test) was used to see where these significant variances lied. Finally, a Chi-Squared contingency table test was performed in order to see if there was an association between seed type and the seed fate at the end of the trial (if it was ignored, handled but not eaten, partially eaten or eaten). The 95% confidence intervals were used on the tests and statistics are reported as means \pm SD.

Results:

We found statistically significant differences for the latency times in function of seed type ($F_{(2, 54)} = 4.65$, $p = 0.01$). Differences for latency time, between *M. racemosum* and *Helianthus sp.* were statistically significant (diff = -171.37, $p = 0.01$). However, differences on latency times between *Helianthus sp.* and *P. biflorum* were statistically insignificant (diff = 128.47, $p = 0.08$) as well as latency times between *P. biflorum* and *M. racemosum* (diff = -42.89, $p = 0.74$). Sunflower seeds had the shortest latency time (89.74 ± 111.45), followed by *P. biflorum* (218.21 ± 190.28), and *M. racemosum* had the longest latency time (261.11 ± 22.88).

For the amount of seeds of each type eaten there were also statistically significant results ($F_{(2, 57)} = 337.3$, $p < 2 \times 10^{-16}$). Differences on amount of seeds eaten between *Helianthus sp.* and *P. biflorum* were statistically significant (diff = -9.30, $p < 0.001$) as well as the differences on amount of seeds eaten between *Helianthus sp.* and *M. racemosum* (diff = 8.95, $p < 0.001$). On the other hand, these differences were not statistically significant between *P. biflorum* and

M. racemosum (diff = -0.35, $p = 0.67$). *Helianthus sp.* had the largest amount of eaten seeds (9.75 ± 1.12) followed by *M. racemosum* (0.8 ± 1.58) and *P. biflorum* had the smallest amount of seeds eaten (0.45 ± 1.10).

For the fate of the seeds at the end of the trials the results were also significant ($\chi^2 = 502.01$, $df = 6$, $p < 2.2 \times 10^{-16}$). The seeds that were ignored the most were *P. biflorum* (53%), followed by *M. racemosum* (45%) and lastly *Helianthus sp.* (2%). The *M. racemosum* was the species mice handled without eating the most (90%) followed by *P. biflorum* which was the one they handled without eating the least (10%). On the other hand, the type of seed that was partially eaten the most was *M. racemosum* (52%) and the one that was partially eaten the least was *P. biflorum* (48%). Lastly, the seed they ate the most was *Helianthus sp.* (89%) followed by *M. racemosum* (7%) and *P. biflorum* had the smallest amount of seeds eaten (4%).

Discussion:

Before starting the experiments with the fresh seeds, we started using dry (mature) seeds of *P. biflorum* and *M. racemosum*. Since we noticed that mice were not eating them, we decided to add dry *Helianthus sp.* seeds (that we know they eat) to show that they were responding adequately to the starvation period. After adding these seeds, mice ate the *Helianthus sp.* seeds but kept rejecting the *P. biflorum* and *M. racemosum* seeds.

With this in mind, we decided to change the experimental design and instead of giving them dry seeds, previously collected fresh seeds were provided. Mice expressed a preference for fresh *P. biflorum* and *M. racemosum* seeds during the new trials over the dry *P. biflorum* and *M. racemosum* seeds given previously (which they rejected consistently). Even when only 6 trials were run with the dry seeds, we observed that there is something about the dry seeds that *P. maniculatus* do not like. Generally, effects of fruits secondary compounds are overlooked and it

makes the understanding of fruit-frugivore interactions difficult. Secondary metabolites in immature fruits act as a defense against predators (Cipollini 2000). The rejection of the dry seeds could be explained by fresh seeds being obtained from unripe fruits. It could be that the ripe fruits contain secondary compounds that have not developed on the unripe fruit yet. A common pattern observed on ripening fruits is the loss of most of their secondary compounds as well as the appearance of novel ones, for example, the case of grapefruits (*Citrus sp.*) (Ortuno et al. 1995; Cipollini 2000).

We found statistically significant differences for latency times between *M. racemosum* and *Helianthus sp.* ($F_{(2, 54)} = 4.65$, $p = 0.01$). Differences on latency times between *Helianthus sp.* and *P. biflorum* were statistically insignificant ($F_{(2, 54)} = 4.65$, $p = 0.08$) as well as latency times between *P. biflorum* and *M. racemosum* ($F_{(2, 54)} = 4.65$, $p = 0.74$). Although these were not statistically significant, deer mice noticed *P. biflorum* in a shorter period of time that they noticed *M. racemosum*. We think this could have been due to the *P. biflorum* seeds being out of the fruit, which made their scent stronger and some rodents use scent cues to locate food (Howard and Marsh 1970).

The results were statistically significant for mice seed type preferences ($F_{(2, 57)} = 337.3$, $p < 2 \times 10^{-16}$). They preferred *Helianthus sp.* seeds to *M. racemosum* and *P. biflorum* ($p < 0.001$). This seems natural since *Henthalius sp.* does not occur at UNDERC and mice display neophilia (the attraction for new things) and tend to try novel foods (Sheppe 1966). On the other hand, they interacted, tried and ate the *M. racemosum* seeds more than they did with the *P. biflorum* seeds (even when these results were also statistically not significant). This supports our hypothesis that *P. maniculatus* would prefer *M. racemosum* over *P. biflorum*.

It is interesting that they noticed *P. biflorum* faster than *M. racemosum* but still had more interaction with *M. racemosum* than with *P. biflorum*. This could be due to the presence of the anthraquinone on *P. biflorum* which is toxic (Flora of North America Editorial Committee 2002). Anthraquinones are known to be mutagenic and may cause cancer in humans. Additionally, there is evidence that some anthraquinones are carcinogenic in rodents (Patel et al. 1989). Although mice can mitigate the effects of secondary compounds such as tannins by acclimation (Shimada et al. 2006), this experimental scenario did not provide the time for acclimation. On an ideal situation, mice could be given a proper acclimation period to these seeds to see if their preferences change.

The two hypotheses were supported by our results: (1) *Peromyscus maniculatus* ate the seeds of the herbaceous plants and (2) preferred *M. racemosum* over *P. biflorum*.

Nonetheless, there is still a question that needs to be addressed: who eats the seeds of these plant species? There is still insufficient information on the anatomy or composition of *P. biflorum* and *M. racemosum*. In addition, research about the secondary compounds present (not only on fruits of herbaceous plants) before and after fruit ripening is also important since it could be one of the factors that affect seed predation. In conclusion, research needs to be done on predators and dispersers of the seeds of herbaceous plants since there are few studies about it. This could eventually lead to new discoveries since this area has not been fully explored.

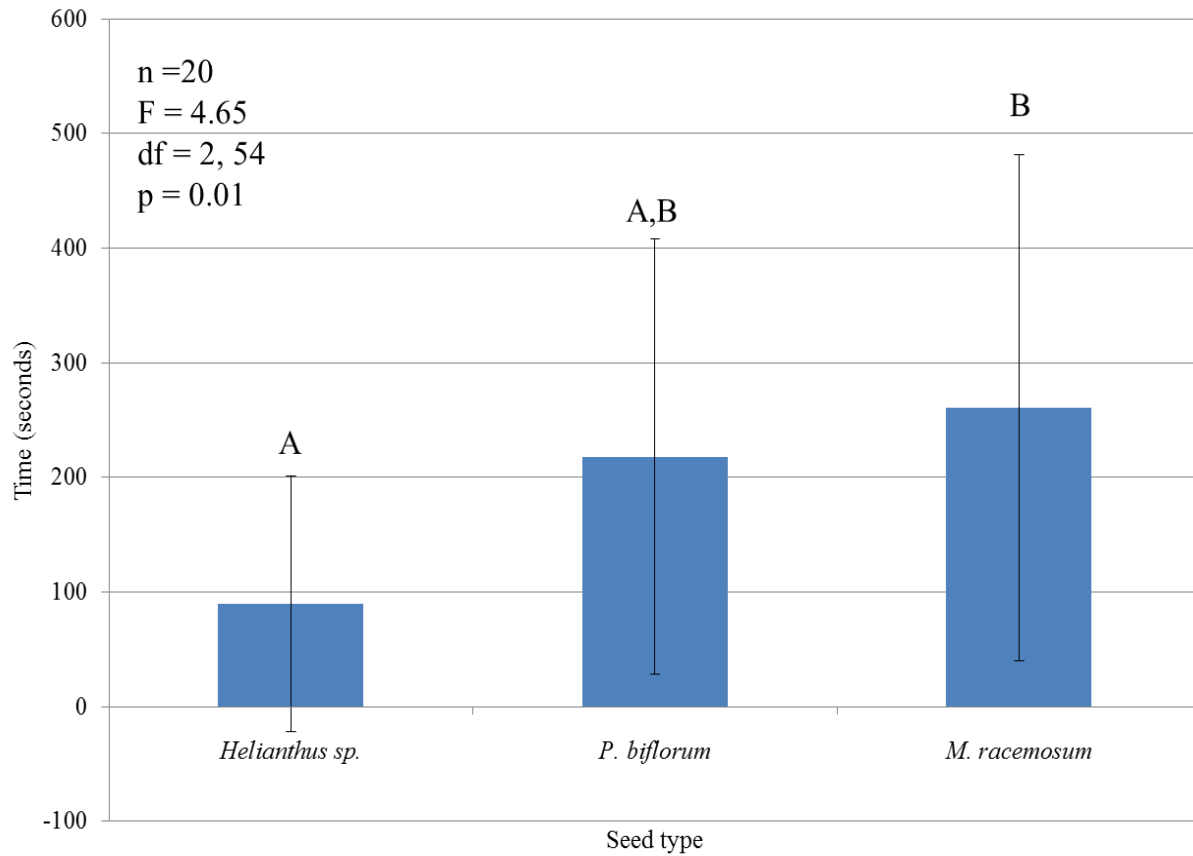
Figures:

Figure 1. Differences on latency times in function to seed type. This figure shows the results of the one-way ANOVA and the post hoc Tukey test. Differences for latency time, between *M. racemosum* and *Helianthus sp.* were statistically significant (diff = -171.37, $p = 0.01$). Differences on latency times between *Helianthus sp.* and *P. biflorum* were statistically insignificant (diff = 128.47, $p = 0.08$) as well as latency times between *P. biflorum* and *M. racemosum* (diff = -42.89, $p = 0.74$).

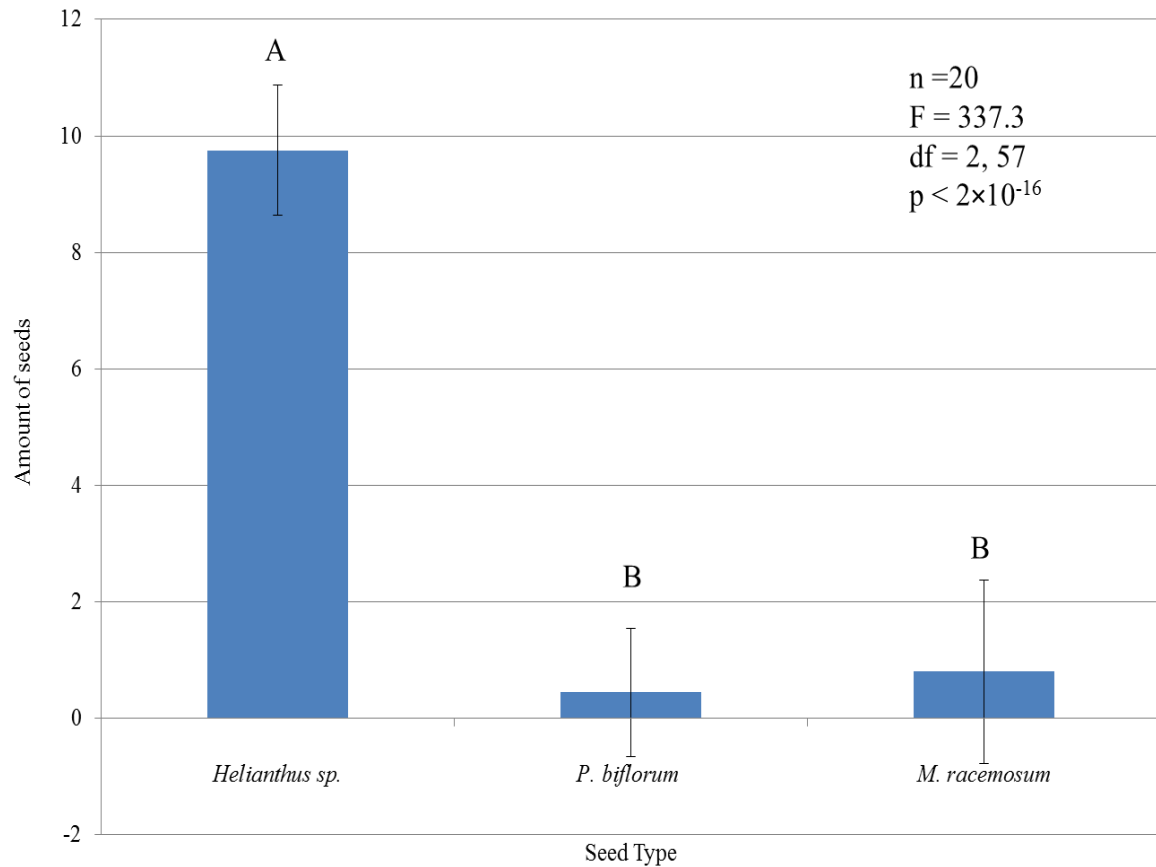


Figure 2. Differences on amount of eaten seeds in function of seed type. This figure shows the results of the one-way ANOVA and the post hoc Tukey test. Differences on amount of seeds eaten between *Helianthus sp.* and *P. biflorum* were statistically significant (diff = -9.30, p < 0.001) as well as the differences on amount of seeds eaten between *Helianthus sp.* and *M. racemosum* (diff = 8.95, p < 0.001). On the other hand, these differences were not statistically significant between *P. biflorum* and *M. racemosum* (diff = -0.35, p = 0.67).

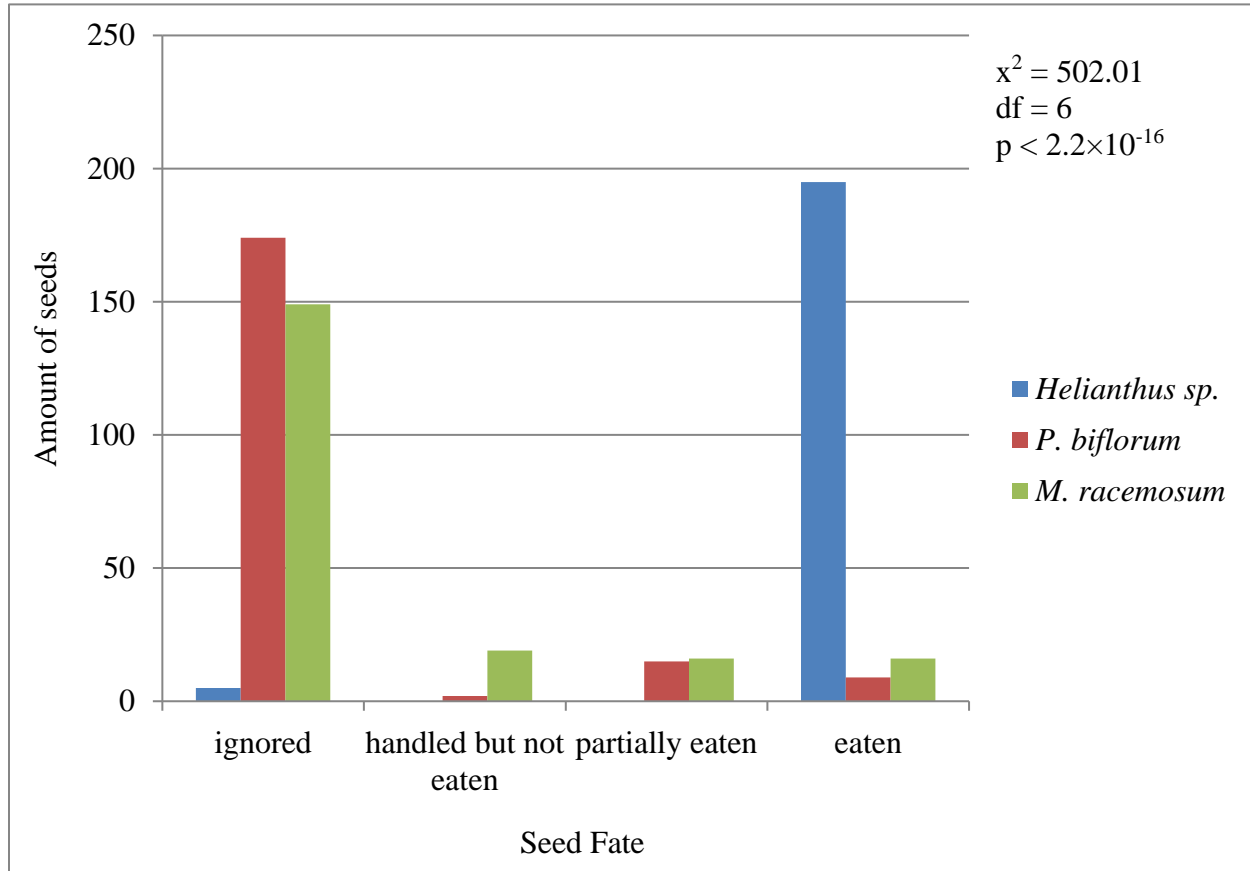


Figure 3. Seed fate at the end of the trials in function of seed type. This figure shows the results of the Chi-Squared test. Results were significant for the seed fate at the end of the trial depending on the type of seed.

Acknowledgements:

I would like to give special thanks to my mentor, Dr. Cramer for being patient and helping me out with every difficulty I faced throughout the realization of my project, helping me with the statistics of my project, and being there every time I needed his help or advice. This short space would not be enough to describe how grateful I am. I would also like to thank Erin Stewart for her help while setting the grids and traps, collecting berries, checking the traps, and housing the mice. Thanks as well to Xiomary Serrano and Kiana Lee for also helping out in the field. My most sincere thanks to Andrea Parra Saldívar and Shirley LeClair-Bernal for driving me around property, helping me collect berries, staying up late with me while I ran my trials, and all their support during my research. Thanks as well to Gary Belovsky, for giving me the opportunity to be here at UNDERC, and to The Bernard J. Hank Family Endowment and to the José Enrique Fernández Endowment for funding my research and stay at UNDERC and making all of this possible.

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